[illegible] INVESTIGATION ON THE ORGANO-TOXIC ACTION OF [illegible], SEBACONITRILE AND LAURYL NITRILE.

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The studies to be reported deal with the morphologic manifestations of the organic effects of two dicarboxy-acid nitriles (sebaconitrile and adiponitrile) and of one aliphatic acid nitrile (lauryl nitrile). An examination of the crude forms of the adiponitrile and lauryl nitrile is included in these investigations.

Adiponitrile.

1. Pure Adiponitrile.

A. Oral Administration.

1. Single Dose:

There were 34 rats in this series. The individual dose administered ranged from 0.0002 cc. per gram to 0.0009 cc. per gram of body weight. Twenty-four rats died from the effects of the chemical given within a period of from 4 hours to 2-1/2 days. Ten rats were killed, 5 after 4 days following introduction and 5 after 7 days following intraoral medication. All surviving rats had received the lowest dose (0.0002 cc. per gram) recorded. The fatalities were irregularly distributed as to dose given.

(a). Eighteen of the rats died within the first

twenty-four hours following treatment: 3 rats, 0.0[illegible] cc. per gram, 4 hours; 2 rats, 0.000[illegible] cc. per gram and 3 rats, 0.000[illegible] cc. per gram, 16 hours; 2 rats, 0.0003 cc. per gram, 1 rat, 0.0004 cc. per gram, 1 rat, 0.0005 cc. per gram, 1 rat, 0.0007 cc. per gram, 18 hours; 2 rats, 0.0004 cc. per gram, 1 rat, 0.0005 cc. per gram, 1 rat, 0.0006 cc. per gram, 20 hours.

Gross Pathology: The internal organs were more or less markedly congested. Hemorrhages were seen in the lung and in the gastric mucosa (pyloric region). Testes of subnormal size were found in 3 rats, which appeared to be of immature development.

Microscopic Pathology: Small multiple perivascular hemorrhages were occasionally present in the brain. The lungs were markedly congested and often contained focal areas of edema and hemorrhage. In one lung multiple hyaline thrombi were observed in the interalveolar capillaries, associated with desquamation of round, vesicular cells into the alveolar lumina. The bronchial lumina contained a moderate amount of a purulent exudate in 2 instances. The liver was always markedly hyperemic and showed in the rats surviving for the longer periods, pericentral vacuolar degenerations. The spleen was usually in a highly congested state, reaching in cases a hemorrhagic appearance. Erythrophagia was extensive and frequent. Brown pigmented phagocytes were sometimes observed in great number in the pulp. There were occasionally perifollicular hemorrhages present. The pulp often showed a marked hyperplasia of the reticulum cells. Atrophy and central necrosis of follicles were not infrequently seen. The kidney revealed varying degrees of tubular degeneration, which may, however, be in part a post mortem phenomenon. Mild to moderate regressive lesions were found in the majority of the testes, consisting in arrest of spermatogenesis, desquamation and vacuolization of spermatids and occasional appearance of spermatid giant cells. The stomach was always normal, with the exception of

ers, in which the suggestion of a superficial glan- lar degeneration existed. All other organs were m. . or less markedly hyperemic.

(b). Seven rats examined died within 26 hours to 60 hours following the oral administration: 1 rat, 0.0002 cc. per gram, 26 hours; 1 rat, 0.0005 cc. per gram, 28 hours; 2 rats, 0.0008 cc. per gram, 2 days; 2 rats, 0.0009 cc. per gram, 2 days; 1 rat, 0.0007 cc. per gram, 2-1/2 days.

Gross Pathology: The internal organs were congested. The lungs, moreover, showed hemorrhagic areas. Small hemorrhagic erosions were present in the pyloric mucosa of the stomach. In 2 rats necroses of the postpyloric duodenal region were present, which had resulted in one instance in a duodenal perforation.

Microscopic Pathology: The brain was normal in most cases. One rat showed, however, multiple hemorrhages, congestion and a few small glia cell foci. The lungs were markedly congested and contained extensive hemorrhagic and edematous areas. Purulent bronchitis existed in 4 cases. The livers were hyperemic and in one case a mild pericentral degeneration existed. The section of one of the stomachs showed a large coagulation necrosis in the glandular mucosa of the stomach. The spleen revealed always a markedly hyperemic pulp associated with a more or less extensive hyperplasia of the reticulum cells and atrophy of the follicles. The tubular epithelium was degenerated to a mild or moderate degree in most instances. Arrest of spermatogeneris existed in the testes of 5 rats, while a medullary hemorrhage was found in the suprarenal of one rat. The other organs were essentially normal with the exception of circulatory changes and post mortem autolysis present in several pancreas.

(c). Five rats received 0.0002 cc. per gram and were killed 4 days after oral treatments, while an additional 5 rats treated with the same dose were sacri-

fixed 7 days subsequent to administration by mouth.

Gross Pathology: The autopsies did not render any pathologic observations with the exception of a perforation of the duodenum with subphrenic abscess under the surface of the liver in one instance.

Microscopic Pathology: Multiple small, perivascular hemorrhages were present in 2 brains. The lungs were normal or moderately hyperemic. In one lung extensive atelectatic areas, intermingled with pneumonic foci were found. The liver was normal in all rats except 2, in which regeneration and degeneration respectively of liver cells was present. The spleen was always more or less markedly congested and revealed in 3 cases extensive erythrophagia. The kidneys showed congestion and mild to moderate tubular degeneration present in 7 instances, once combined with tubular invaginations into the Bowman's capsule spaces. Arrest of spermatogenesis associated with mild degeneration of the spermatogenic epithelium of the testis existed in 6 rats.

2. Repeated Doses:

There were 6 rats in this series. They received 0.0001 cc. per gm. 3 times per week for 6 weeks and were killed at the end of this period.

Gross Pathology: The lungs were congested and showed a few fresh hemorrhages, apparently caused by the inhalation of ether. The testes of 1 rat were grey and soft. All other organs were grossly normal.

Microscopic Pathology: The lung of 1 rat contained an encapsulated abscess with pneumonic tissue in the environment. In a second rat there was a cellular thickening of the interalveolar septa and extensive atelectasis, while purulent bronchitis and localized pneumonia existed in the lung of a third rat. Mild degeneration of the liver cells was found once. The spleen was usually congested and revealed extensive erythrophagia. Degeneration of the tubular epithelium of the kidney was found in 4 cases, 3 times associated with tubular invaginations of the Bowman's capsule

spaces. Localized marked degeneration of the spermogenic epithelium of the testis existed in only 1 rat. All other organs, including the stomach, were normal.

Comment: The acute local effect resulting from oral administration of pure adiponitrile, expecially frequent with the higher doses (above 0.0003 cc. per gram), consisted in hemorrhagic necroses (erosions) of the glandular or pyloric portion of the stomach and of the post-pyloric part of the duodenum. In the latter area they sometimes caused perforation followed by localized peritonitis and subphrenic abscess formation.

The acute systemic action was manifested in circulatory and vascular disorders. There existed general congestion of the internal organs, especially of the lung, liver and spleen. In lethal cases there was edema and hemorrhages of the lung, which apparently predisposed to the inflammatory conditions of the pulmonary parenchyma and bronchi seen in cases dying not quite acutely. The engorgement of the pulp of the spleen was usually excessive and showed sometimes a hemorrhagic character. The presence of frequent erythrophagia in the spleen suggested increased erythrocytic destruction, while the atrophy and central necrosis seen in the splenic follicles may be the result of the circulatory disturbances prevailing in this organ. These disorders were evidently also the cause for the regressive lesions existing in the liver, kidney and testes of an appreciable portion of the animals. All these changes were apparently of rather

treatment return, if the animal survived, as they were either present or present in a mitigated form in those mice which were killed 4 and 7 days after treatment.

The lesions observed in the rats given repeated but small doses were essentially identical with those seen after a single larger dose. The acute circulatory and vascular disorders were, however, less manifest in this series and the lesions of a degenerative type were in the foreground.

B. Inhalation:

1. Single Exposure:

Pathologic data were available from 6 rats and 6 mice. Three of the rats were exposed to a concentration of 0.28 mg. per liter for eight hours and then killed, while a second group of 3 rats had been exposed to a concentration of 0.59 mg. per liter for 8 hours before they were sacrificed.

Three of the mice died during the exposure after inhaling the vapors of a concentration of 0.28 mg. per liter for from 3 to 7 hours. A fourth mouse died 24 hours following an 8 hour inhalation of this type, while fifth was killed at this time. The sixth mouse of this series had been exposed to a concentration of 0.59 mg. per liter for 8 hours and was killed 24 hours later.

Gross Pathology: The liver and spleen of the rats showed moderate congestion. The mice which died during the

exposure had hyperemic lungs, livers and spleens, while these organs were pale in the mice killed [illegible]. The paleness was apparently caused by the method of death, that is bleeding from the jugulary veins and carotid arteries.

Microscopic Pathology: The histologic changes seen in the organs of the rats of the 2 groups were practically identical and consisted in more or less marked congestion of the internal organs. There were minor scattered degenerations of the liver cells and of the tubular epithelium of the kidney, sometimes associated with early tubular invaginations into Bowman's capsule spaces. The muscular tissue of the heart showed isolated hyalinized bundles of muscle cells. Erythrophagia was seen in the engorged pulp of the spleen. The brain contained congested vessels, and a few hemorrhages in one rat. Marked unilateral degeneration with calcification of the testis existed once.

The lungs of the mice which died showed diffuse hemorrhages and purulent bronchitis. The internal organs were congested. The myocardium contained a moderate leucocytic and mononuclear infiltration. The organs of the mice which were killed were essentially normal with the exception of fresh traumatic hemorrhages in the lungs of all mice and a purulent bronchitis in one.

2. Repeated Exposures. Pathologic data were obtained from 6 rats and 6 mice. The rats inhaled a concentration of 0.5 mg. per liter for 6-1/2 hours daily, 5 times per week. One rat died after two weeks of this treatment, another was killed after 3 weeks and the remaining 4 were sacrificed after 4 weeks.

The six mice were killed at the end of an identical type and period of exposure (4 weeks).

Gross Pathology: The spleen was markedly congested in the rat with spontaneous death. An enlarged spleen of dark red color and internal viscera of a peculiar brown

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color and eyes with subretinal hemorrhages were found in the rat killed after 3 weeks' exposure. The organs of the other rats were essentially normal.

The post mortem examination of the mice showed grossly normal organs.

Microscopic Pathology: The lungs of one rat showed thickened cellular interalveolar septa, while a purulent bronchitis was present in a second rat. One liver contained pericentral leucocytic foci with surrounding liver cell degeneration. Erythrophagia, brown pigmentation and atrophic follicles were seen, each once. The renal tubules revealed a mild to moderate degree of degeneration in 4 rats. A large subretinal hemorrhage was observed in the eye of one rat. Testicular degenerations of moderate to marked degree were found in 2 rats.

The histologic examination of the organs of the mice revealed minor and inconstant degenerative lesions in the liver and mild but constant degenerative changes in the tubular epithelium of the kidneys. All other organs were essentially normal with the exception of the testes of one mouse with spermatid giant cells in moderate number.

Comment: The circulatory effect in the animals with spontaneous death was manifested in congestion of the internal organs. There was a noteworthy frequency of myocardial lesions in rats and mice after single exposures. Pulmonary changes, except of the circulatory congestive disturbance, were found in several instances (mice) showing purulent bronchitis. Renal degenerations of nephrotic type were observed in the animals after single and repeated exposures. The hepatic and testicular lesions were in general too inconstant for definite evaluation.

C. Subcutaneous Injection:

Ten rats received 0.0001 cc. per gram subcutaneously. 44

Five of them were killed after 4 days and the other 5 rats after 7 days following the injection.

Gross Pathology: Negative with the exception of small testes in one rat. The skin and subcutaneous tissue were normal.

Microscopic Pathology: There was a moderate congestion of the internal organs present. An inflammatory interstitial reaction with leucocytic thrombi was seen in the lung of one rat. Myocardial degenerations associated in one case with small fibrotic foci were observed twice. The most constant and definite changes occurred in the kidney. They were of a degenerative type and usually of moderate degree, being combined with tubular invaginations and hemorrhages into Bowman's capsule spaces in one instance each. Mild testicular regressive changes were found 3 times, while they were marked and diffuse once.

Comment: The subcutaneous injection of pure adiponitrile apparently did not cause any local reactions at the site of injection. It is, however, doubtful if this is generally the case. The systemic lesions were degenerative changes in the kidneys and possibly minor regressive changes in the heart and testes.

D. External Cutaneous Applications:

Six rats and 8 rabbits were used in this investigation. The adiponitrile was applied to the skin in amounts of 1 cc. daily. The possibility existed, however, that the animals would inhale the chemical or lick it and thus introduce it into the stomach.

Three rabbits received the same amount to the skin, which had been injured by several small cuts. The other 5 rabbits received applications on the intact skin.

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Of the rats, one died after the first application, another one after the second treatment, 2 after the third, one after the fourth and the sixth after and the fifth treatment on the seventh day of the experiment.

One of the rabbits with cut skin died 10 hours after the second application; another one died after the third treatment and the third one survived 12 applications given 3 times weekly during a period of 4 weeks. It was then killed. One of the rabbits in which the chemical was applied to the intact skin died 10 hours after the second application, another one died after 9 treatments received during 3 weeks at the rate of 3 applications per week, and 3 rabbits which had been exposed to the same treatment for 4 weeks were killed at the end of this period.

Gross Pathology: The internal organs of the rats, especially the lungs and hearts were congested. The stomachs of practically all animals were distended with gas and showed hemorrhagic areas in the pyloric region. There was a perforation of the duodenum near the pylorus present in 2 rats, in one of which it was associated with generalized peritonitis. The kidneys were pale brown.

The pathologic inspection of the rabbits to whose cut skin the adiponitrile was applied showed in one rabbit extensive post-mortem decomposition. In the rabbit which died after 3 applications the skin revealed ulceration in the area of application with widening of the cuts. The ulceration involved the subcutaneous connective and muscle tissue with extensive pus formation. The left lung was congested and the liver and spleen were slightly hyperemic. The skin and internal organs of the rabbit which survived 4 weeks of this treatment were normal.

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The autoptic examination of the other 5 rabbits with applications to the uncut skin did not reveal any definite local reactions and minor to moderate congestion of the internal organs.

Microscopic Pathology: The rat which died after one treatment showed moderate hyperemia of the internal organs, mild nephrosis and increase and proliferation of the reticulum cells in the spleen, which contained follicles with degenerated centers. The skin revealed a certain localized thickening of the epidermis. In the rats which died later there were localized areas of epidermal atrophy and necrosis. Cellular debris covered the surface of small ulcers and of markedly atrophic areas. The subepidermal tissue showed necrosis of a hyaline type. In several instances the lung was highly hyperemic and edematous. The stomach mucosa showed, in one instance, a large hemorrhagic necrosis of the glandular portion in one rat. The spleen was often atrophic and had a fibrotic pulp containing brown pigment. The tubular epithelium of the kidney was more or less markedly degenerated. There was arrest of spermatogenesis present in the testes of all rats. Spermatid giant cells were found in several instances in appreciable number. Immature desquamated spermatogenic epithelial cells filled the ducts of the epididymis.

The skin of the 2 rabbits which died spontaneously after applications to the cut skin showed a degeneration and extensive necrosis respectively of the epidermis. The subepidermal vessels were markedly dilated and hyperemic. Hemorrhages and edema associated with leucocytic infiltration and fibroblastic proliferation were observed in one instance. The lungs contained a diffuse hemorrhagic edema with degeneration and desquamation of the alveolar epithelial cells. The spleen had an empty pulp and small follicles. There was an extensive tubular degeneration and necrosis present in the kidney. The glomerular spaces were dilated and filled with cellular debris from disintegrated invaginated tubular epithelium.

The skin of the rabbit which survived the treatment revealed only a mild subepidermal lymphoid infiltration. The liver had numerous degenerated liver cells. The kidney was markedly hyperemic and showed tubular degeneration. Scattered areas of myocardial degeneration

were found in the heart. The brain was normal.

The skin of the 2 rabbits which received applications on the intact skin and died during the course of the experiment showed atrophy of the epidermis and marked edema of the subcutaneous tissue and necrosis and fibrosis of the muscle tissue. The lung was markedly hyperemic and diffusely edematous, while small myocardial degenerations occurred in the heart. The liver was edematous, contained localized hemorrhages and necroses surrounded by dense leucocytic zones. The kidneys revealed marked degeneration of the cortical tubules with frequent invagination (tubuli) into the distended Bowman's capsule spaces. The organs of 2 of the surviving 3 rabbits of this series were essentially normal. In the third rabbit which was killed at the end of the 4 weeks' period the skin showed atrophy and ulcerative defects with marked epidermal hyperplasia at their edges. Fibrous granulation tissue forming the floor of the ulcers extended deep into the muscle tissue. There were groups of multinucleated giant cells in the subcutaneous tissue a mild generalized lymphoid infiltration and proliferated and dilated capillaries. The hair follicles nearby showed sproutings of the epithelium and the formation of several hairs from the same follicle. The germinative cells appeared to be swollen. The other organs were without any appreciable abnormalities with the exception of a few blanched areas indicating myelin degeneration in the brains of these 3 rabbits.

Comment: The observations made indicate that adiponitrile may not only injure intact epidermis of rats and rabbits, but apparently also penetrates through it and causes vascular disturbances and degenerative lesions and necroses in the tissue underneath extending even into the muscle tissue. The effects are aggravated when the skin is damaged by cuts made previous to applications of the chemical. The internal organs show the direct and indirect effects of the circulatory and vascular disorders produced by the adiponitrile. The organs are hyperemic and often

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edematous and degenerative lesions occur in various organs, such as liver, kidney, spleen, heart, testes. The ulcerations of the gastric mucosa present in the rats provide evidence that at least part of the adiponitrile applied to the skin was licked off and thus entered the stomach, causing the previously recorded gastric mucosal defects.

II. Crude Adiponitrile.

A. Oral Administration:

Six rats received a single dose of crude adiponitrile by mouth. The dose varied between 0.0006 cc. to 0.0007 cc. per gram. The animals died within 12 to 20 hours after this administration.

Gross Pathology: Congestion of the internal organs and small hemorrhagic erosions in stomach.

Microscopic Pathology: The internal organs showed dilated and engorged vessels. There was interstitial edema present in the hyperemic liver in several rats. The lungs revealed hemorrhagic edema. There was some post mortem degeneration present in the kidney.

Comment: The rapid death following the administration did not allow for any severe morphologic cellular changes. The pathology present was of circulatory and vascular type, indicating severe disturbances in the circulation of blood, and tonus and permeability of vascular walls.

B. External Cutaneous Applications:

Two rabbits received applications of 1 cc. of crude

adiponitrile 3 times weekly for 5 weeks. They were killed at the end of this period.

Gross Pathology: No particular changes of the skin. Internal organs normal.

Microscopic Pathology: The skin and internal organs were essentially normal with the exception of a mild tubular degeneration in the kidney of one rabbit. Clumps of pyknotic nuclei were found in the lumina of the excretory tubules.

Four rats were treated on the skin with 1 cc. of crude adiponitrile for 5 days and were killed on the seventh day.

Gross Pathology: The rats were emaciated. The lungs, liver, and spleen were markedly hyperemic. One rat had small left testis with yellow dots.

Microscopic Pathology: The skin was normal in all rats with the exception of one, which showed localized atrophy of the squamous pithelium. Mild nephrotic changes sometimes associated with tubular invaginations were found in all rats. Minor regressive lesions were also present in the spermatogenic epithelium of the testes. These were, however, of a much more severe type in one rat, where a diffuse testicular degeneration with numerous intratubular calcifications was seen. The liver of this animal showed, moreover, a diffuse granular and vacuolar degeneration of the liver cells. Erythrophagia was observed in the spleen and the heart contained scattered hyaline degenerative foci.

Comment: A toxic action of the crude adiponitrile on the skin of rabbits and rats was absent under the experimental conditions applied. The mild degenerative changes seen in the kidney and testes of many animals suggest, however, that the material penetrates through the skin and exerts a systemic effect. It

may be possible that the severe organic changes present in one rat indicate the nature of lesions which would appear after more severe and prolonged treatment.

General Comment

The local action of pure adiponitrile and to some extent also of crude adiponitrile upon skin and mucous membranes (stomach, duodenum), is represented by degenerative and necrotic changes whenever this action is intense and prolonged enough. It seems to be possible that vascular and circulatory disturbances set up in the sub-mucosal tissue contribute to the injurious effect exerted upon the epithelial tissue above. The adiponitrile, in contrast to the previously studied diamines, appears to have a greater affinity for the glandular parts of the stomach than to the squamous cell portion, as the hemorrhagic erosions observed after intraoral administration were always located in the pyloric part and also in the postpyloric portion of the duodenum.

The systemic action of this compound is mainly one of vascular and circulatory nature, the blood vessels of the internal organs being dilated, engorged and permeable to the liquid and corpuscular components of the blood. These conditions, apparently in conjunction with a direct action of the chemical upon the cells of the various organs, being probably of metabolic character, are responsible for the regressive

lesions found in the various organs, especially the kidneys, spleen, liver, heart, testes and occasionally the brain also.

It stands to reason that the vascular and circulatory phenomena may also have played a part in the development of the acute inflammatory and probably infectious conditions in the subcutaneous tissue of the rabbits treated by skin applications and of the purulent bronchitis and pneumonic lesions observed not infrequently in animals of other series.

Sebaconitrile

A. Oral Administration:

Twenty-three rats received oral feeding of 0.0001 cc. to 0.0010 cc. per gram. All animals died spontaneously within 40 minutes to 30 hours after the treatment. Eighteen died within the first 5 hours; 5 died 20 to 30 hours after introduction of the chemical.

Gross Pathology: There was some unabsorbed material in the stomach of those rats which died very acutely. The internal organs were highly congested. Hemorrhages were found in the lungs and heart. In several instances there was some bloody mucus in the stomach and duodenum. The gastric mucosa was normal.

In the 5 rats which died later the internal organs were hyperemic, mainly the lungs and heart, less the liver and spleen. Hemorrhagic erosions were found in the pyloric part of the stomach of 2 rats.

Microscopic Pathology: The internal organs of all rats were more or less markedly congested. The lungs usually showed in addition to vascular engorgement diffuse edema and scattered extensive hemorrhages. Purulent bronchitis existed once and atelectatic

areas occurred in 2 rats. Degeneration of the liver cells, either of diffuse hydropic type or of scattered focal character, was seen in 9 rats. Scattered necrotic areas sometimes of large size were found in the pancreas of 11 rats. This condition was associated with interstitial hemorrhages and leucocytic infiltration in 2 instances each. In the stomach of 2 rats localized hemorrhagic necroses were observed in the glandular mucosa. Three other rats showed, on the other hand, localized areas of atrophy of the squamous epithelium. The intestinal mucosa of one rat was found to be necrotic. The tubular epithelium of the kidney was in almost all rats more or less markedly swollen, degenerated or necrotic. The Bowman's capsule spaces were distended and filled with a hyaline, granular matter, mixed in one instance with erythrocytes, in 4 rats. A moderate regenerative, partly atypical regenerative proliferation of the tubular epithelium was seen in 3 rats.

Summary: The local effect of sebaconitrile is manifested by the hemorrhagic necrotic foci found in the glandular portion of the gastric mucosa of several rats and the more diffuse necrosis of the intestinal mucosa of one rat. The systemic action is evidently of a vascular and circulatory nature, causing engorgement of the vessels of the internal organs followed by edema and hemorrhages such as seen in the lungs of many rats and in the pancreas of several animals. A definitely toxic effect is also exerted upon the tubular epithelium of the kidney, giving rise to an excretion of an albuminous and occasionally also erythrocytic urine. The regenerative tubular proliferations are, however, not connected with the chemo-toxic action, as they were seen in rats which died within a few hours after the oral administration of the chemical. As these animals did not show

any evidence of an infection, the cause of these lesions remains undetermined.

Lauryl Nitrile.

I. Pure Lauryl Nitrile.

A. Oral Administration:

1. Single Dose:

There were 29 rats in this series. They received doses ranging from 0.001 cc. per gram to 0.014 cc. per gram. Seventeen of them died spontaneously, the other were killed. Nine of the rats died within the first 24 hours after treatment. Three rats died during the second day, while 6 rats were killed during this period. Five of the rats died from 3 to 6 days after the treatment, while 6 were killed during this period.

Gross Pathology: The rats which died spontaneously showed a more or less marked congestion of the internal organs. The lungs were hyperemic and often edematous and hemorrhagic. The stomach was frequently distended with gas and the contents had the typical odor of lauryl nitrile. Many of these animals had hemorrhagic erosions in the congested pyloric, glandular portion of the stomach and free blood occurred in the lumen. In the rats dying during the first day, blood was sometimes also found in the intestine. A similar gastric condition existed also in many of the rats which were killed during the early experimental period. In the rats which died or were killed after a few days there were necroses and ulcers in the duodenum and often perforations which had produced periduodenal abscesses or localized peritonitis involving the posterior surface of the liver. The spleen and liver were often congested and increased in size.

Microscopic Pathology: In the 9 rats which died during the first 24 hours after the treatment vascular and circulatory phenomena were most prominent. The brain

of several rats was hyperemic and contained numerous small perivascular hemorrhages. Small glia cell foci were present in the subcortical zone of one brain. The lungs were extremely congested and edematous and showed smaller and larger hemorrhagic areas. A purulent pleurisy associated with inflammatory foci in the pulmonary parenchyma existed in one case. The liver revealed also a status of passive congestion combined in 2 cases with interstitial edema and in 3 instances with mild degenerative changes in the liver cells. Focal necroses were found in the pancreas of 4 rats, while multiple interstitial hemorrhages were observed once. Hemorrhagic smaller and larger necroses were seen in the glandular mucosa of the stomach of 3 rats, while local degenerative and atrophic lesions existed in the squamous epithelium of the cardiac gastric portion in 2 cases. Necrosis of the intestinal mucosa existed in one animal. The pulp of the spleen was markedly engorged and had a hemorrhagic character in one instance. There was an increase of the reticulum in 3 rats and evidence of erythrophagia in one rat. Follicular degeneration was seen in 2 rats. The tubular epithelium always showed degenerative changes, which were marked in 5 rats, moderate in one and mild in 3 rats. Tubular invaginations into Bowman's capsule spaces were found in the kidney of one rat. The testicular germinative parenchyma revealed mild regressive lesions.

While vascular and circulatory disturbances were still striking in the animals which died or were killed on the second day after the treatment, they were less marked than those found in the group just described. The brain was often hyperemic and contained in 5 cases multiple hemorrhages. Glia cell foci and perivascular satellitosis was observed once. The lungs were moderately congested and showed focal hemorrhages in one instance. The hepatic vessels were engorged. The squamous cell mucosa of the stomach revealed in 2 rats a moderate hyperplasia of the epithelium with evidence of hyperkeratosis. A small ulcer was seen in this region in one animal, while atrophic degenerative changes of the squamous cells occurred in another animal. The pulp and sinuses of the spleen were engorged and there was an increase of the reticulum cells present. Erythrophagia and follicular degeneration were round once each. The kidneys showed only mild regressive lesions of the tubular epithelium. The mucosa of the urinary

bladder on one rat possessed a swollen epithelial lining associated with edema of the submucosa. Mild testicular degenerations were generally present.

In the rats which died or were killed subsequent to the above mentioned animals, that is 3 to 7 days after treatment, the brain contained moderately often perivascular hemorrhages. The lung was usually congested and showed in the rats which died spontaneously atelectases, hemorrhages, abscesses, inflammatory foci, thrombosis of pulmonary vessels with surrounding leucocytic infiltration and purulent pleurisy. The vascular bed of the liver was engorged in all animals. There were, in some cases, irregularities in the stainability of liver cells, while in one rat with spontaneous death several large anemic necroses were found. The pancreas of the spontaneously dying rats revealed hemorrhagic lesions, multiple small abscesses, phlebitis and purulent peripancreatitis. The gastric mucosa showed atrophic, degenerative as well as hyperplastic changes in the squamous epithelial portion. The squamous epithelium was in several instances papillary, hyperkeratotic and showed in places also invasive qualities. A small ulcer with environmental inflammatory reaction was seen in the squamous epithelial part in 2 rats. A purulent perigastritis was found once. The mucosa of the duodenum had foci of hyaline necrosis in 2 instances. The spleen of the rats which died showed, in several cases, atrophic and hyaline changes in the pulp. In the other animals the pulp was congested and contained an increased number of reticulum cells occasionally associated with erythrophagia. Mild to marked regressive lesions were found in the tubular epithelium of the kidney. They were associated in 3 instances with tubular invaginations into Bowman's capsule spaces. A regenerative proliferation of the tubular epithelium producing an atypical variety of epithelial cells was seen once. The testicular regressive changes were usually of mild nature. Only twice they attained moderate to marked degrees. The bone marrow was often hyperemic and sometimes even hemorrhagic. In one of the latter cases there was an atrophy of the myeloid elements, while a second marrow of this type contained numerous immature myeloid elements and very scanty mature ones.

2. Repeated Doses:

One series of rats received 0.0008 cc. per gram of pure lauryl nitrile 3 times weekly. One of them died after the third experiment, while 4 others were killed after a treatment period of 6 weeks. The rats of the second series received 0.0002 cc. per gram 3 times a week for 6 weeks and were killed at the end of this period.

Gross Pathology: The animal which died did not show any perforation of the duodenum. The autoptic examination of the other rats was negative as to pathologic lesions.

Microscopic Pathology: The main and most important changes present in the rats of both series were found in the stomach. There was, in several animals, a hyperplastic papillary and hyperkeratotic thickening of the equal epithelium. A small ulcer in this region was found once. The submucosa was edematous and mildly to moderately densely infiltrated with neutrophilic and eosinophilic leucocytes. Perivascular hemorrhages of the brain were present in 3 rats. Inflammatory processes existed in the lungs of 2 rats. Interstitial edema and granular degeneration of the liver occurred once. The spleen was congested and showed erythrophagia in 4 instances. Degenerative changes of the renal tubular epithelium attaining always only a mild degree were found 5 times. They were associated with tubular invaginations into Bowman's capsule spaces in 3 instances. Hemoglobinous casts and calcium casts were found once each. Marked testicular degeneration occurred only once in a rat with a gastric lesion.

Comment: The acute action of orally introduced pure lauryl nitrile is local as well as general in character. The local effect is mainly exerted upon the mucosa of the stomach, causing there hemorrhagic ulcers in the glandular parts, accompanied by hemorrhage into the gastro-intestinal tract. The systemic effect

is characterized by severe vascular and circulatory disturbances resulting in passive congestion of the internal organs, especially the lung, liver and spleen, followed by edema and hemorrhages in various organs (lung, brain, pancreas, spleen). Degenerative and necrotic lesions may be subsequent to these alterations (pancreas, kidney, testis). In animals surviving for several days, degenerative and inflammatory phenomena become more prominent. They are, in certain instances, associated with regenerative cellular proliferations. In the stomach there occur then also degenerative changes in the squamous epithelial parts combined with submucous edema, leucocytic infiltration and not infrequently also ulceration. On the other hand also proliferative changes are observed here, evidenced by the appearance of papillary hyperplasia of the squamous cells, hyperkeratosis and occasionally also infiltrative growth of the epithelial cells into the submucosa. The prolonged effect upon the duodenal mucosa caused by a retention of part of the introduced substance in the stomach, results, in a considerable percentage of the rats, in the development of duodenal ulcers followed by perforation and the formation of subphrenic abscesses, involving the liver and pancreas.

Reactive phenomena in the spleen following the congestive state are characterized by increase of reticulum cells, degeneration of the lymph follicles and finally also atrophy of the pulp.

While the liver lesions were in general rather mild in regard to the parenchymal elements, there were occasionally also more extensive necroses present being apparently the result of the serious localized circulatory disturbances in the liver. Similar thrombotic lesions were seen a few times in the lung and pancreas. The presence of glia cells foci and perivascular satellitosis in some of the brains attested that the systemic action of the chemical may sometimes also cause more severe degenerative changes in this organ. Hemorrhages and atrophic changes in bone marrow found in animals surviving the treatment for a few days indicate also that there may occur reactive phenomena in the blood forming organs. The frequent incidence of erythrophagia in the spleen of treated rats suggests moreover that there is also an increased destruction of erythrocytes going on in the spleen of these animals. This may have been responsible for the observation of hemoglobinous casts in the kidney of one rat. The changes present in the rats receiving repeated administrations of smaller doses were in general identical with those seen in the rats which survived for several days a single treatment with a larger dose.

B. External Cutaneous Application.

Four rats received daily cutaneous applications of 1 cc. pure lauryl nitrile. Two of them died after the fourth treatment. The surviving two rats were killed on the seventh day of the experiment following 5 treatments.

Four rabbits received 3 times weekly applications of 1 cc. pure lauryl nitrile. Two were killed after 3 weeks of this treatment. The other 2 animals were killed at the end of the fourth week.

Gross Pathology: The 2 rats which died were markedly emaciated. The lungs were mildly congested. The liver of one rat showed grey spots. The testes of the 2 rats which were killed later were small and had yellow dots.

The organs of 3 rabbits were essentially normal. The skin of one rabbit, killed after 4 weeks of treatment, showed a small superficial ulceration in the area of application. The internal organs were normal.

Microscopic Pathology: The skin of one of the rats with spontaneous death showed a purulent blister. The brain was hyperemic and contained hemorrhages and glia cell foci in 2 rats. Regenerative activity of the liver cells was present in the congested liver. The kidney was markedly nephrotic in one of the rats with spontaneous death and revealed moderate tubular degeneration in another rat. One of the rats showed mild testicular changes, another moderate ones and a third marked degenerative lesions. The bone marrow of one rat contained numerous nucleated erythrocytes and erythrocytic debris.

The skin of all 4 rabbits revealed definite and in part marked degenerative changes. The epidermis was in places atrophic and covered with a necrotic leucocytic coat. In 2 rabbits purulent blisters and small ulcerative defects occurred. There were moreover hyperkeratosis and papillary hyperplasia present in one instance associated with the formation of intraepidermal cysts filled with cornified pearls. A mild to moderate eosinophilic leucocytic infiltration was found in the edematous submucosa. Mild hyaline myocardial degenerations occurred in the hearts of 3 rabbits. The livers were markedly congested in 2 rabbits and degenerative tubular lesions existed in the kidneys of 3 rabbits.

Comment: The prolonged cutaneous application of pure lauryl nitrile causes degenerative and hyperplastic epidermal changes which may be accompanied by blister formation and production of hornified cysts. The systemic manifestations are similar to those seen after oral administrations, with a somewhat longer survival period. They are, however, in general, less marked after cutaneous application.

C. Subcutaneous Injection.

Sixtee rats received a subcutaneous injection of 0.01 cc. per gram. Six were killed after 3 days, 5 rats were sacrificed after 4 days, and the surviving rats were killed after 7 days.

Gross Pathology: The rats killed 3 days after injection showed, at autopsy, only general congestion of internal organs, apparently caused by the ether anesthesia used. The rats sacrificed after 4 days had a slight local inflammatory reaction at the site of injection, while the last group of rats showed cutaneous lesions containing a yellow purulent matter. The internal organs were grossly normal.

Microscopic Pathology: The brains of 3 rats in each of the 3 groups showed perivascular hemorrhages. They were associated in one instance with a small focus of liquefaction necrosis surrounded by a glia cell infiltration and in another case with satellitosis. Inflammatory changes occurred in only 2 rats, one having a purulent bronchitis and the second one showing a localized interstitial pneumonia. The liver was markedly hyperemic in the rats killed after 3 days. The condition was associated in 2 rats with mild degenerative alterations of the liver cells. Marked passive congestion often associated with erythrophagia was found in the same group of rats. The squamous epithelium of the stomach of 2 rats was thickened, while vacuolar degeneration of these cells with submucous edema and leucocytic infil-

tration occurred in another rat. The kidneys were increasingly congested with the lengthening of the time of exposure. Tubular degenerations and intracapsular tubular invaginations were not infrequently present. Hemoglobinous casts were found once. The skin of the rats killed after 4 and 7 days showed abscess formation and epidermal necrosis. There was a reactive fibroblastic and leucocytic reaction in the surrounding tissue.

Comment: Subcutaneously introduced pure lauryl nitrile causes a somewhat slowly developing inflammatory reaction in the subcutaneous connective tissue accompanied by necrosi and ulceration of the epidermis and connective tissue. The systemic effects produced by the resorbed chemical is as already pointed out vascular and circulatory, especially during the first 3 days following injection, as evidenced by marked hyperemia of the brain and spleen. Erythrophagia in the spleen and the presence of hemoglobinous casts in the kidney suggest a hematotoxic action. The nephrotoxic effect is identical with that seen following other routes of introduction. The occurrence of degenerative and hyperplastic changes in the squamous epithelium of the stomach cannot be clearly related to the treatment administered. While it may be possible that they may represent the results of a systemic vascular disturbance, it appears more likely that some of the injected substance may have leaked out of the skin and have been licked off and thus may have entered the stomach directly.

D. Inhalation:

1. Single Exposure:

Six rats, 3 of them exposed for 8 hours to 2.05 mg. per liter and 3 to 0.75 mg. per liter were killed 15 hours after treatment.

Fifteen mice were exposed to 1.31 mg. per liter for 8 hours. One mouse died during the exposure and 4 others were killed after the treatment; 5 were killed 24 hours later, while the remaining 5 were sacrificed 3 days following exposure.

Gross Pathology: The rats showed at autopsy enlarged dark red livers and spleens. The mice were normal.

Microscopic Pathology: The internal organs of rats and mice were markedly hyperemic. Mild to moderate nephrotic lesions were found in the kidneys of the rat, often associated with tubular invaginations into Bowman's capsule spaces. Two rats showed perivascular hemorrhages in the brain. The organs of the mice were normal except for the previously mentioned circulatory changes.

Comment: With the exception of minor degenerative lesions in the kidneys of the rats and circulatory changes in the internal organs no injurious effect was observed in rats and mice exposed to the inhalation of lauryl nitrile vapors for a period of 8 hours.

2. Repeated Exposures:

Six rats were exposed for 7 hours daily during a period of 4 weeks to the inhalation of pure lauryl nitrile. They were killed at the end of this period.

Six mice were subjected to a similar exposure for a period of 4 weeks and then killed.

Gross Pathology: Negative

Microscopic Pathology: Three of the rats had multiple hemorrhages in the brain. The lungs of the rats were hyperemic and one had a purulent bronchitis and focal pneumonia. Liver and spleen were in several instances markedly hyperemic. Marked erythrophagia was observed in the pulp of the spleen. The kidneys showed mild degenerative changes of the tubular epithelium and occasionally tubular invaginations into Bowman's capsule spaces. Hemoglobinous matter was observed in the tubular epithelium and lumina of 4 rats, while erythrocytic debris in the same location was associated with this condition in 2 rats. The organs of the mice were more or less hyperemic. Degenerative tubular lesions in the kidney occurred in some of the mice.

Comment: The repeated and prolonged exposure of rats and mice to the inhalation of pure lauryl nitrile causes some congestion of the internal organs. The most striking and important lesions were found in the kidney, in which the tubular degeneration was complicated by the excretion of hemoglobinous matter and erythrocytic debris, indicating that an increased destruction of erythrocytes is taking place during the exposure. The marked frequency and incidence of erythrophagia in the spleen of the rats supports this conclusion.

II. Crude Lauryl Nitrile.

A. Oral Administrations:

1. Single Dose:

Seven rats received oral medication of crude lauryl

nitrile in doses ranging from 0.004 cc. per gram to 0.014 cc. per gram. Three rats died within 24 hours, the other 4 rats died 4 to 5 days later.

Gross Pathology: The lungs were congested and contained hemorrhagic areas. The stomach mucosa was swollen and hyperemic and a coffee-ground colored matter was found in its lumen. The intestines were brownish and edematous and contained a brownish watery or hemorrhagic matter. The liver and spleen were swollen and congested. A purulent diffuse peritonitis existed in one rat.

Microscopic Pathology: The internal organs were markedly congested. The lungs were hyperemic and edematous and contained hemorrhagic areas. A small ulcer was present in the squamous cell portion of the mucosa of one rat, while hemorrhages existed in the surrounding mucosa. A purulent gastritis was seen in a second rat. The hyperemic liver contained, in several rats, liver cell degenerations and necroses. A purulent perihepatitis was seen once. Scattered necroses were seen in the pancreas. The spleen showed in 4 instances atrophy of the pulp and central necrosis of the lymph follicles. A hyperemia of the pulp existed in 3 rats. The tubular epithelium of the kidney was mildly to markedly degenerated or directly necrotic. Tubular invaginations into Bowman's capsule spaces were observed once. The tubular epithelium was markedly swollen.

Comment: The organic lesions observed after oral administration of crude lauryl nitrile are essentially identical with those seen after the introduction of the pure compound.

B. External Cutaneous Application:

Six rats received daily applications of 1 cc. of crude lauryl nitrile. One died after 4 applications, while the other 5 were killed after 5 treatments on the seventh day of the experiment. Two rabbits received the same treatment 3 times weekly

for 3 weeks and were then killed.

Gross Pathology: The internal organs were hyperemic. The rat which died spontaneously was emaciated. A marked subcutaneous infection with purulent, cheesy exudation was seen in one rabbit.

Microscopic Pathology: The skin of the rats showed dilated hair canals filled with the debris of disintegrated hairs. The skin of one rabbit revealed a marked hyperplastic thickening and hyperkeratosis with purulent intraepidermal blisters and subcutaneous fibrosis. The cutaneous changes of the second rabbit were more severe. In addition to a papillary hyperplasia of the epidermis with the formation of cornified cysts and thickening of the epithelium lining the hair canals, there were numerous abscesses present in the subcutaneous tissue. The lungs of the rat which died spontaneously were congested and edematous. The stomach of another rat showed multiple subepithelial abscesses with epithelial defects in the squamous epithelial portion. The submucosa was edematous and infiltrated with leucocytes. Mild to moderate tubular degeneration was found in the kidney of some of the rats and of both rabbits. The other organs did not reveal any appreciable pathologic alterations except some variations in their blood count.

Comment: The observations made show that the cutaneous application of crude lauryl nitrile to the skin of rats and rabbits causes degenerative inflammatory and hyperplastic lesions. The morphologically similar condition found in the stomach of one rat is evidently the result of the accidental introduction of the chemical into the stomach, by licking of the skin to which the chemical was applied. The systemic effect is demonstrated by the occurrence of the kidney lesion.

C. Subcutaneous Injection:

Two rats which had received 0.01 cc. per gram and

0.016 cc. per gram, respectively, by subcutaneous injection, died within 24 hours after injection and 14 days respectively.

Gross Pathology: The lungs of the rat which died acutely were congested and the brain was markedly hyperemic. The lungs of the second rat contained hemorrhagic areas in a slightly brown parenchyma. The liver and spleen were markedly enlarged. The kidneys were purplish red, the suprarenals dark red and there existed retroperitoneal edema.

Microscopic Pathology: The internal organs of the rat which died within 24 hours after injection were congested. Hemorrhages were present in the lungs. Phagocytes loaded with brown pigment were seen in the pulp of the spleen. A mild degeneration of the renal tubules was observed.

The lungs of the rat which died later showed thickened and cellular interalveolar septa and interstitial tissue containing a leucocytic infiltration. The liver was markedly congested and a moderate, diffuse degeneration of the liver cells existed. The Kupffer cells were increased in number and swollen. The sinusoids contained myeloid elements. Strands of hyperchromatic liver cells were scattered in the parenchyma. The spleen was congested and contained numerous proliferated reticulum cells, megakaryoblasts and myeloid cells. Erythrophagia was frequently seen. The follicles were very small. The suprarenals were markedly hyperemic and hemorrhages involved the medulla. Mild degenerative tubular changes were observed in the kidney. The retroperitoneal tissue in the right kidney region contained a large abscess walled off by an inflammatory fibroblastic capsule which extended into the adjacent liver where degeneration of liver cells, hemorrhages, leucocytic infiltration and fibrosis with multinucleated giant cell formation were present.

Comment: The rat which died soon after the subcutaneous injection showed the usual organic manifestations of the acute circulatory and vascular effect of the lauryl nitrile in addition to the nephrotoxic action exerted by this chemical. The second

rat revealed the conditions observed repeatedly before, following a chronic exposure to lauryl nitrile, that is, locally a progressive necrotizing inflammatory reaction which lead apparently to the formation of the retroperitoneal abscess, being an extension of the changes set up in the subcutaneous tissue at the site of injection, and systemically degeneration of the liver, atrophy of the follicles in the spleen, erythrophagia in the pulp and possibly also chronic interstitial pneumonia.

D. Inhalation:

Twenty-eight mice were examined which had been exposed to the inhalation of lauryl nitrile vapors released from the crude product. Five died during the inhalation, while thirteen were killed directly after the treatment which consisted in exposure lasting for six hours. Two were killed nine days after inhalation, five, 10 days later and three, 12 days following inhalation. The doses given varied in concentration between 0.12 mg. per liter to 2.6 mg. per liter.

Gross Pathology: The lungs were hyperemic in the 5 mice which died during the exposure. The post mortem examination of the other mice was negative as to pathologic lesions.

Microscopic Pathology: The trachea studied in the group of mice dying during inhalation showed acute purulent inflammation. The lungs contained some fresh hemorrhages and in several instances purulent bronchitis and focal parenchymal inflammations in addition to congestion. Degenerative changes in the liver cells were present in a few instances. Perifollicular hyalinization and follicular central necroses were found in the spleen of

several animals. Only one kidney showed mild regressive lesions of the tubular epithelium.

The organs of the mice killed 9 to 12 days after the treatment were in general normal with the exception of necrotic foci occurring in the liver of some of them. They were partly of hyaline character. Hyaline changes were occasionally observed in the spleen.

Comment: The pathologic lesio observed in the inhalation series were of rather mild type. During the stage of acute action there seems to be some irritation of the trachea and lung present. These changes were, however, only impressive in the group of animals which died spontaneously and which was exposed to the highest concentration used.

Whether or not the degenerative and necrotic lesions found in the livers of mice killed several days after the treatment are referable to the chemical action is somewhat doubtful, as lesions of similar character are often the result of infectious conditions of incidental nature.

General Comment

The local effect of lauryl nitrile upon the skin and mucous membranes of the stomach and duodenum is similar to that seen in animals treated with adiponitrile. It is, however, evident from the observations made that a more prolonged exposure of the gastric mucosa to the chemical does not only result in the production of hemorrhagic ulcers in the glandular portion of the stomach and duodenum, but also in the squamous cell portion of the stomach, where moreover marked hyperplastic epithelial reactions

may occur. The resorption of subcutaneously introduced lauryl nitrile is apparently a relatively slow one causing thereby a rather late appearing necrotizing inflammation with a prolonged progressive tendency. Oral administration is apparently the most dangerous exposure, if judged by the extent and character of the pathologic organic lesions produced, and inhalation seems to be, as far as definite organic injury is concerned, the least hazardous one, unless an acutely lethal dose is given. The chemical produces systemically not only vascular and circulatory disturbances, but also direct cellular damage in the brain, liver, spleen and pancreas and seems to affect, under certain conditions, also the erythrocytic elements and the bone marrow.

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